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Optimization of media parameters for the enhanced production and activity of lipase by bacterial lipase isolates

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ABSTRACT:
Bacterial lipase isolates, B1, B3 & B4 were investigated for better lipase production and activity through media optimization parameters involving nutrient broth containing oil of varying concentration, different nitrogen source and pH. The culture media was prepared as nutrient broth containing 1% oil emulsified with pH adjusted to 7. This culture media was varied with oil concentration in the range of (1-10) % and the nitrogen source (1%) was varied as yeast extract, peptone, NH_4Cl and $NaNO_2$; whereas pH was varied in the range of 5-9 separately. The oil content varied in the study by groundnut oil for isolates B1 & B3 and by olive oil for isolate B4 as per the optimized carbon sources of the respective isolates. In the results, bacterial lipase isolate B4, emerged as the best isolate with maximum lipase activity of 42 U/ml using optimal media parameters as 3% olive oil emulsified in nutrient broth, 1% NH_4Cl & pH 8, along with other optimized parameters of 30^oC, 150 rpm and 24hrs culturing period. The higher oil concentrations in the culture broth led to decreased lipase activity, which may be due to the substrate (oil) inhibition of microbial growth leading to the reduced lipase production levels. Hence, the bacterial lipase isolate B4 may be further studied for its possible use in the commercial lipase production and for the research in various frontiers of biotechnology.

Keywords: Lipase, Media Optimization, Lipolytic Activity, Substrate Inhibition, Nitrogen Source, Lipase Isolates

INTRODUCTION

Lipase (E.C. 3.1.1.3) is the biocatalyst found in various biological sources on the Earth. It has the versatile catalytic activity in converting higher order lipid molecules to lower order fatty acid molecules in the biodegradation process of recycling lipid molecules among the living and non-living matter of the planet Earth. Though, the lipase is found in all the living organisms, the research has been carried out on its sources to screen & isolate the better lipase producing sources [1] to produce and commercialize the lipase for its reputation in industrial applications. Among the most exploited lipase sources [2] the microbial sources are the most suitable and amicable solution to peruse for the novel and efficient lipases [3-5]. Among such microbial lipase producers, *Bacillus* & *Pseudomonas* had been some of the prominent microbial species to be used for experimental investigations to produce the higher volumes of cost effective & high yielding lipases [1, 6-14].

The best choice for the higher production of lipases had been the submerged cultivation [15], which in turn is dependent on media composition and physical process settings. These physical process conditions are met by the maintenance of temperature, pressure, DO [16-18] etc. but then the composition of media in terms of carbon source [19-20], nitrogen source [21], pH and the mineral salts play a vital role [2, 22,23] in the total fermentation process. So for any submerged fermentation process the media optimization in terms of its basic components and the other physical parameters becomes the major step in the development of production process. This media optimization process would also help in identifying the best carbon source, nitrogen source and other nutrients along with the physiological conditions and compositions for the growth of biomass and the product of interest. Thus the present research work illustrates the media optimization process for the optimal production of lipase using varying carbon source and different nitrogen sources along with the other physical process parameters using the soil bacterial lipase isolates.

MATERIALS AND METHODS

Bacterial lipase isolates

The bacterial lipase isolates were obtained from soil samples by serial dilution method, followed by screening for lipase producing bacteria by streaking suspected colonies [24] on to the trybutyrin agar media followed by streaking on to rhodamine B agar plates for confirmation and isolation of the UV illuminating orange fluorescent colonies [25]. The best isolated lipase producing bacteria were further screened by agar well diffusion method [26-27] and selected them as B1, B3 & B4. These isolates were preserved on agar slants at 4^oC in the refrigerator for further use [28].

Optimization of media parameters

Composition of oil - the carbon source

The nutrient broth media of pH 7 was emulsified with 1%, 3%, 5%, 7% & 10% oil of groundnut oil & olive

oil as carbon source [19-20] and lipase inducer [27, 29] separately and these emulsified media were inoculated with 5% inoculum of 0.5 McFarland overnight grown lipase isolates B1, B3 & B4 respectively and incubated the culture flasks at 30°C & an agitation speed of 150 rpm in an orbital shaker incubator [30]. The broth samples were withdrawn aseptically after 24hrs of culturing and centrifuged at 10,000g & 4°C for 15 minutes. The resultant cell free supernatant was used as crude enzyme and assayed for lipase activity.

Nitrogen source

The culture media of nutrient broth emulsified with 1% olive oil (for isolate B4) and groundnut oil (for isolates B1 & B3) separately were added with 1% nitrogen source like peptone, yeast extract, ammonium chloride, and $NaNO_2$ for each culture and used these media to culture the lipase isolates B1, B3 & B4 keeping all other parameters and procedure same as above.

pH of culture broth

The pH was varied from 5 to 9 in the nutrient media emulsified with 1% olive oil for isolate B4 and 1% groundnut oil for isolates B1 & B3 separately; and the lipase isolates B1, B3 & B4 were cultured by keeping all other parameters and procedures same as above.

Lipase assay

Lipase assay was performed using titrimetric method [31-32] and olive oil as the substrate. The substrate cocktail was prepared with 10% olive oil, emulsified in 5% gum acacia in 100mM phosphate buffer at pH 7. 1ml of the crude enzyme was added to the substrate cocktail and incubated for 15 minutes at 37°C. The enzyme catalytic reaction in the mixture was stopped by addition of 1ml of ethanol-acetone solution (1:1), and the fatty acids were extracted by swirling the contents swiftly. Extracted fatty acids were measured by titrating against 0.05M NaOH solution [33] till the end point of pH 10, using phenolphthalein indicator [22].

One unit (U) of the lipase enzyme was defined as the amount of enzyme required to hydrolyze 1µmol of fatty acids from triglycerides of olive oil. Lipase activity was calculated as micro moles of free fatty acids formed from olive oil per ml of crude lipase enzyme [34] given by (Eqn. 1)

$$Activity = \frac{(V_S - V_C).N.1000}{S} \quad (1)$$

Where, $\mathbf{V_S}$ is the volume of 0.05M NaOH solution consumed by the sample enzyme-substrate cocktail (ml); $\mathbf{V_C}$ is the volume of 0.05M NaOH solution consumed in the titration by the substrate (Control) cocktail (ml); **N** is the molar strength of the NaOH solution used for titration (0.05) and **S** is the volume of substrate cocktail solution (10ml). Hence the activity of lipase enzyme was expressed as U/ml of crude lipase enzyme extract.

RESULTS & DISCUSSION

The effect of oil (substrate) concentration, nitrogen source (1%) and pH for optimal lipase production by isolates B1, B3 & B4 was observed through the experimental study, and the subsequent results were depicted in the Figures 1-9. The optimal lipase activity (16 U/ml) was observed for isolate B1 at the groundnut oil concentration of 5% and 1% NH_4Cl as nitrogen source along with pH 7 of the culture media; whereas isolate B3 produced enhanced lipase activity (25 U/ml) at the same 5% ground nut oil concentration as carbon source and 1% peptone as nitrogen source along with the pH 8.

Similarly, lipase isolate B4 produced the maximum lipase activity, 42 U/ml with optimal lipase production conditions as 3% emulsified olive oil in nutrient broth with 1% NH_4Cl as nitrogen source & pH 8. From the Figures 1, 4 & 7 the increase in oil (substrate) concentration increased the lipase activity indicating the direct proportionality relation between the lipase activity & the substrate concentration.

However after reaching the maximum lipase activity with respect to substrate concentration, the lipase activity started to decline; which may be symbolic to reduced lipase production due to substrate inhibition of microbial growth at higher substrate concentrations leading to the decreased lipase activity [35].

On the other hand, Figures 2, 5 & 8 indicate the profound influence of NH_4Cl and peptone on lipase activity of the isolates B1, B3 & B4. Peptone has augmented [19] the enhanced lipase activity as the second best nitrogen source for isolates B1 (13 U/ml) & B4 (27 U/ml). However for isolate B3, peptone was the best nitrogen source for optimal lipase production & activity.

For isolates B1 & B4, NH_4Cl was found to be the excellent nitrogen source for optimal lipase production and activity. It may be the case that NH_4^+ from NH_4Cl could be directly absorbed in to the cellular system channelizing the NH_4^+ molecule as a precursor for better metabolic activities. It may also be the case that NH_4^+ had been used in stabilizing the lipase structure and hence the enhanced activity [21].

From the Figures 3, 6 & 9 it may also be noted that the maximum lipase activity was recorded for these microbial lipase isolates between pH 7 & 8 indicating the stability and optimal activity of lipase in this pH range (7-8) similar to the findings of microbial lipases reported in the literature [36-38].

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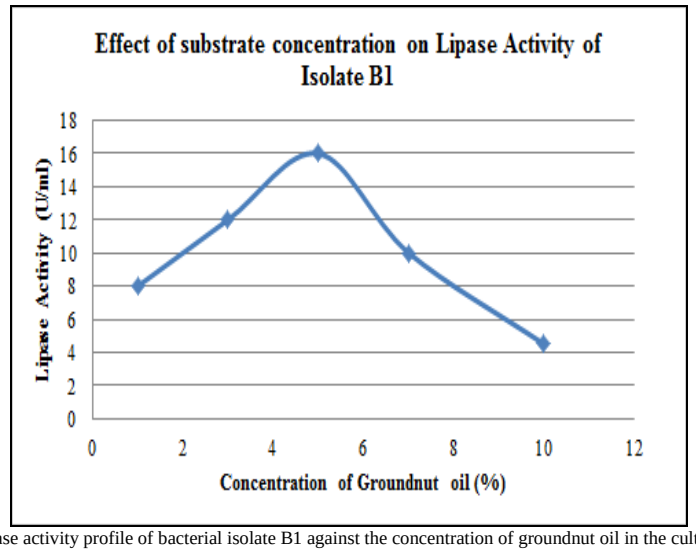

Fig.1 Lipase activity profile of bacterial isolate B1 against the concentration of groundnut oil in the culture media

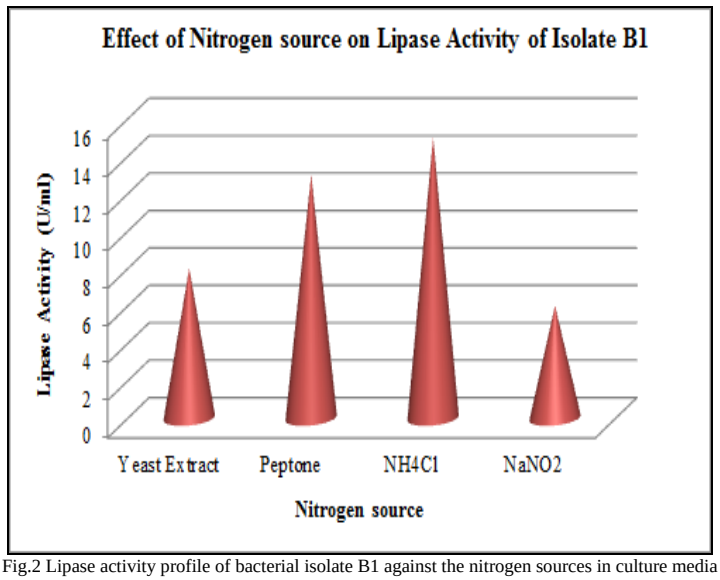

Fig.2 Lipase activity profile of bacterial isolate B1 against the nitrogen sources in culture media

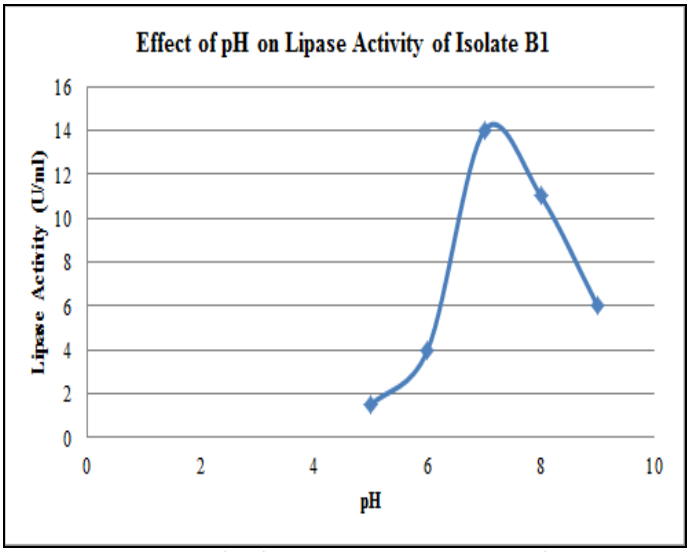

Fig.3 Lipase activity profile of bacterial isolate B1 against the pH of culture media

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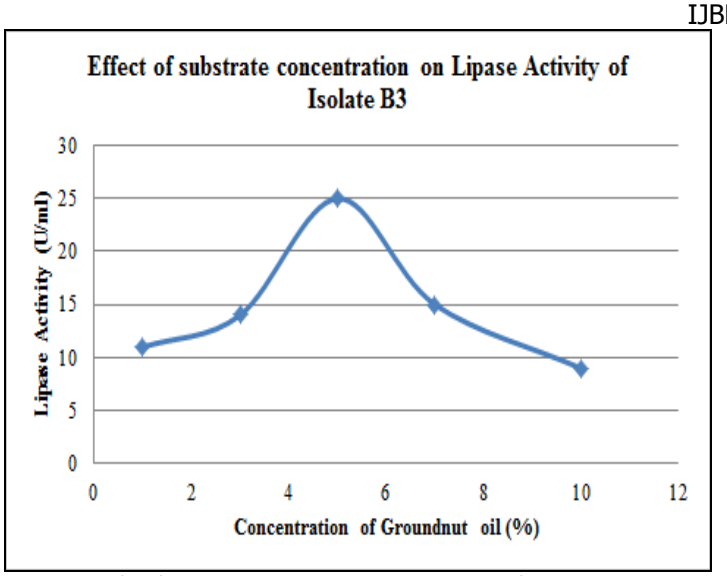

Fig.4 Lipase activity profile of bacterial isolate B3 against the concentration of groundnut oil in the culture media

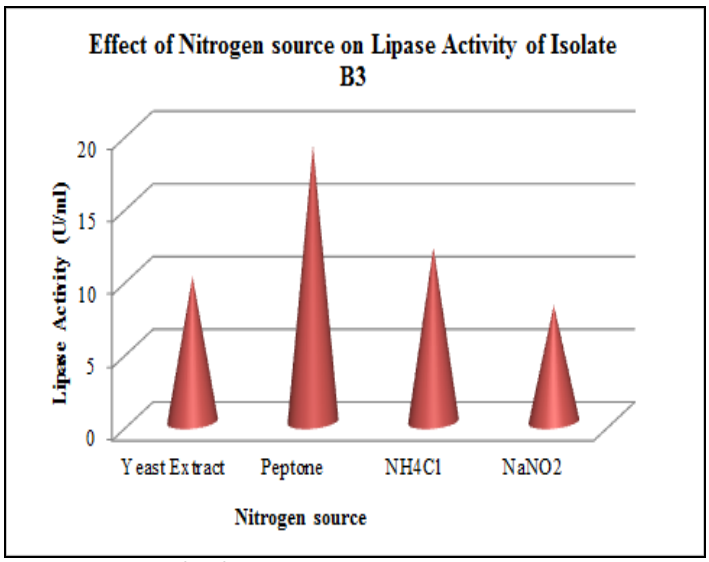

Fig.5 Lipase activity profile of bacterial isolate B3 against the nitrogen sources in culture media

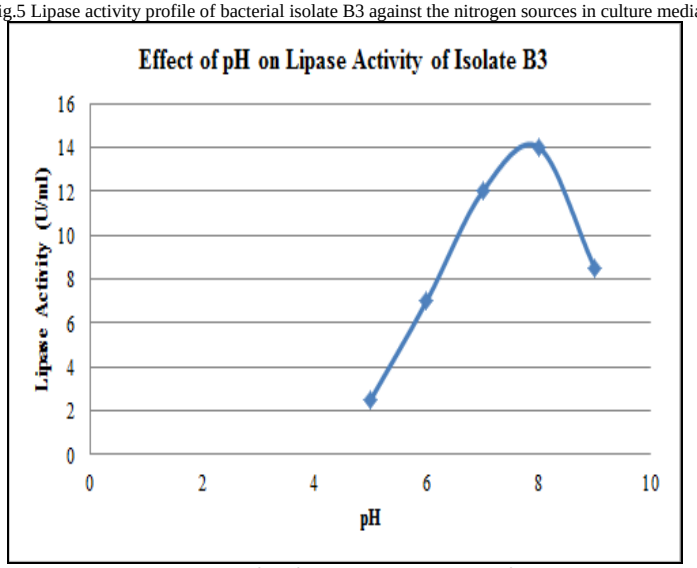

Fig.6 Lipase activity profile of isolate B3 against the pH of culture media

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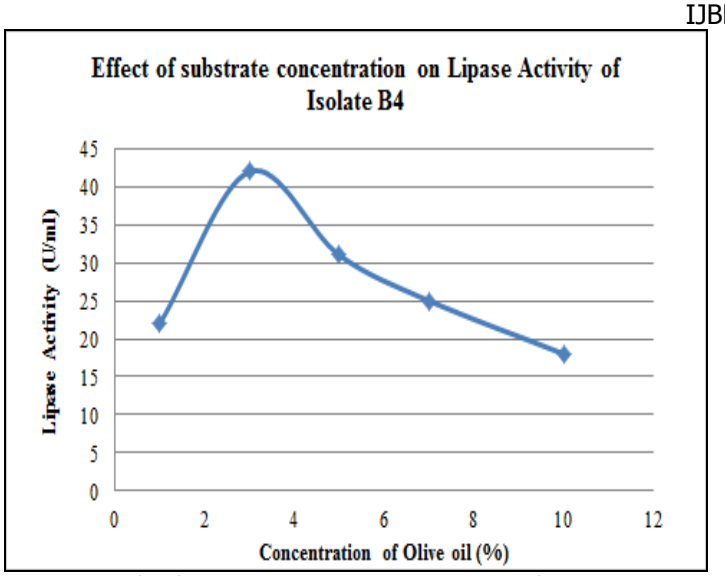

Fig.7 Lipase activity profile of bacterial isolate B4 against the concentration of olive oil in the culture media

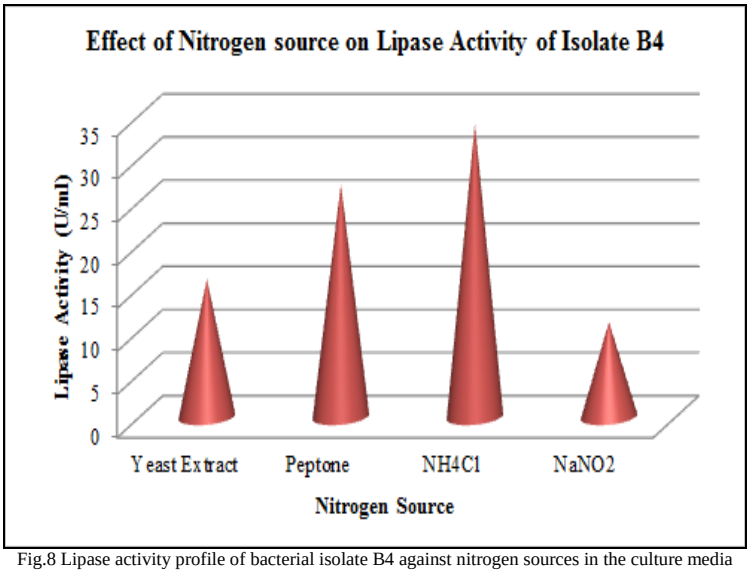

Fig.8 Lipase activity profile of bacterial isolate B4 against nitrogen sources in the culture media

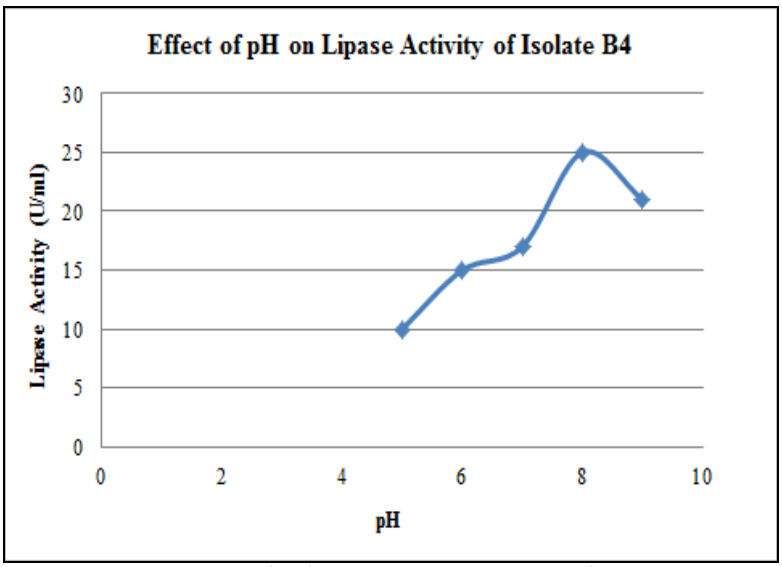

Fig.9 Lipase activity profile of bacterial isolate B4 against the pH of culture media

CONCLUSIONS

Among the bacterial lipase isolates used under the media optimization studies, the isolate B4 has emerged as the best lipase producing isolate with the maximum lipase activity of 42 U/ml. The lipases produced by the isolates B1, B3 & B4 exhibited lower activity levels at higher concentrations of emulsified oil (beyond 3-5%) in the culture media; which may be due to the substrate inhibition of microbial growth at higher substrate (oil) concentrations leading to the reduced lipase production levels. The best optimal lipase production parameters for culturing media were emerged as 3% olive oil, pH 8, with 1% NH_4Cl as nitrogen source in nutrient broth with the other optimal conditions of 30^oC & 150rpm for more than 24hrs of culturing period for isolate B4. Hence the bacterial lipase isolate B4 may be further studied for its possible usage either in commercial lipase production or for the research in various frontiers of biotechnology.

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